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Phenolic Content, Antioxidant Capacity, and Antimicrobial Activity of Essential Oil from *Habbatus sauda* Seed

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Motivation

Nigella sativa commonly known as habbatus sauda belongs to a botanical family of *Rununculaceae*. The *N. sativa* seed is rich with medicinal value and has been used as a natural remedy since ancient time by people in the Mediterranean region. Previous research revealed that it contained an abundance of active ingredients useful for anticancer and anti-inflammatory, anti-dermatophyte, asthma, hypertension, diabetes, cough, bronchitis, fever, dizziness and gastrointestinal disturbances.

The first step to recover and purify essential oil from plant materials involves an extraction process. The yield of essential oil is dependent on the solvent used, extraction method and condition. Conventional extractions such as soxhlet extraction and maceration (ME) are normally performed at high temperatures for several hours. In recent years, a better extraction method has been developed such as the ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical extraction. Supercritical extraction is less favorable owing to its energy consumption and higher capital cost. The localize superheating in microwave-assisted extraction induces a rapid temperature rise thus possesses challenge in temperature control. Extraction is a mass transfer process involving solvent transport to the solid phase (inner transport), dissolution of the solutes (solubility) and release of solutes from the solid matrix to the bulk phase (external transport). The UAE technique reduces the inner and external mass transfer limitation and hence increases the yield of extraction. Furthermore, ultrasonic wave can break the cell membranes reducing control of inner mass transport. Therefore, the UAE method was employed in this work. Solvent type plays important role in essential oil extraction. A combined effect of different extraction methods (ME, and UAE) and varying solvent polarity to the polyphenol extraction from *N. sativa* has never been studied previously, and hence this is the objective of this work.

Methodology, Results and Discussion

Extraction of essential oil from *N. sativa* seeds were performed using ME and UAE. The effect of different solvent (n-hexane, ethanol, methanol, 50% ethanol, 50% methanol) on the composition of essential oil yield was investigated using maceration solvent extraction. The total phenolic (TPC) and flavonoids (TFC) content were analysed using Singleton's method and aluminum chloride colorimetric assay, respectively. Meanwhile, the free-radical scavenging (antioxidant) activity of the extract and essential oil was measured by means of 2,2-diphenyl-1 picryl hydrazyl (DPPH) assay. Gas chromatography mass spectrometry (GC-MS) analysis of the essential oil showed a significant amount of thymoquinone (0.6~0.8%), thymol (0.09~0.15%), agidol 7 (0.33~6.44%), p-cymene (0.31~0.57%), and (E)-β-Ocimene (0.11~0.13%). It was found that extraction of antioxidant (Agidol 7) favours aqueous solvent, i.e. 50% ethanol and 50% methanol (Fig. 1). The highest yield of essential oil was obtained using 50% ethanol followed by n-hexane, while the lowest is 50% methanol. Extraction using ethanol shows highest simultaneous extraction of TFC (2.47 µg QE/g DW), TPC (0.23 mg GAE/g DW) and antioxidant activities (60%). Methanol has a higher yield of TPC (0.38 mg GAE/g DW) and antioxidant activities (67%) but very low TFC (0.25 µg QE/g DW). Extraction using n-hexane, 50% methanol and 50% ethanol is not notable, thus, ethanol was employed for the remainder of this work. It was found that there is no significant difference at $P < 0.05$ on the effect of residence time and ultrasonic frequency after 30 minutes. However, the highest TPC, TFC and antioxidant activities were obtained at power 224W and temperature of 50 °C (Fig. 2). Extraction

at the higher temperature (60 °C) is not an improvement due to thermal degradation of bioactive compounds. It was found that UAE is better than ME as it can provide much higher extraction of TFC, TPC and antioxidant within 30 minutes compared to 4 hours for ME. Fig. 3 shows excellent antimicrobial properties of the essential oil extracted from *N. sativa*, which were studied using *E. coli*. The inhibition area was obtained by processing the image as binary using ImageJ software. The highest inhibition effect of 30.17% was achieved using oil obtained from ethanol extracts, meanwhile both methanol and n-hexane extracts also showed good antimicrobial properties. Oil obtained using aqueous solvent i.e. 50% ethanol showed only 7.25% inhibition, due to absent in thymol and thymoquinone.

Table 1: GC-MS analysis of essential oil from *Nigella Sativa* seeds

VOC	n-Hexane	Ethanol	Methanol	50% Ethanol	50% Methanol	Harzallah et al. (2011)
p-Cymene	0.57	0.43	0.31	ND	ND	49.48
(E)-β-Ocimene	0.13	ND	0.11	ND	ND	ND
Thymoquinone	0.8	0.6	0.63	ND	ND	0.79
Thymol	0.09	0.14	0.15	ND	ND	ND
Agidol 7 (AO 425)	0.33	0.45	1.14	6.44	2.43	ND
Sitosterol	0.05	0.07	0.07	ND	ND	ND

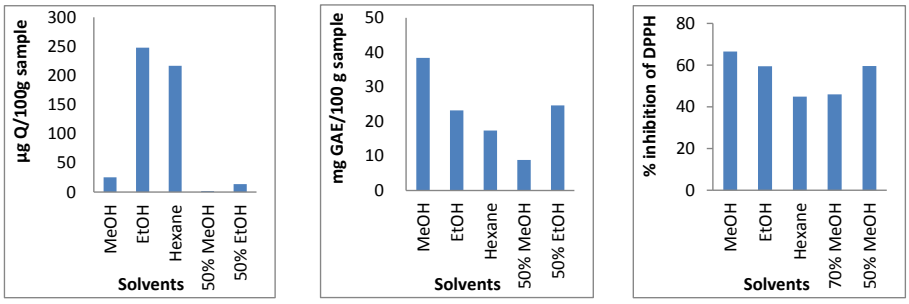

Fig. 1: Effect of solvent to TFC, TPC and antioxidant extraction from *N. sativa*

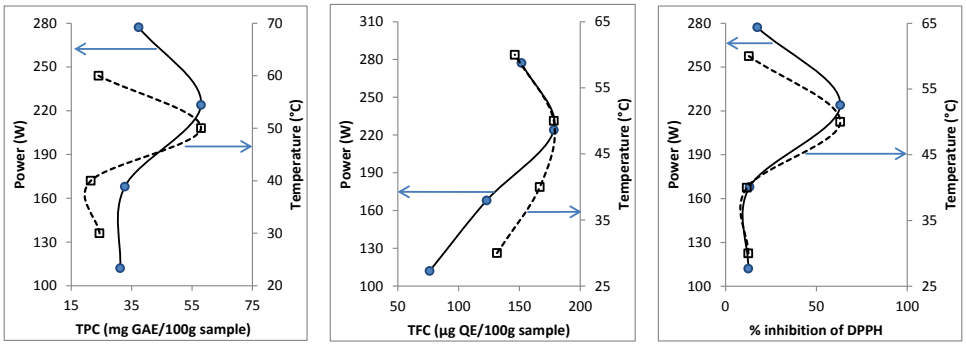

Fig. 2: Effect of power and temperature to TFC, TPC and antioxidant extraction from *N. sativa*

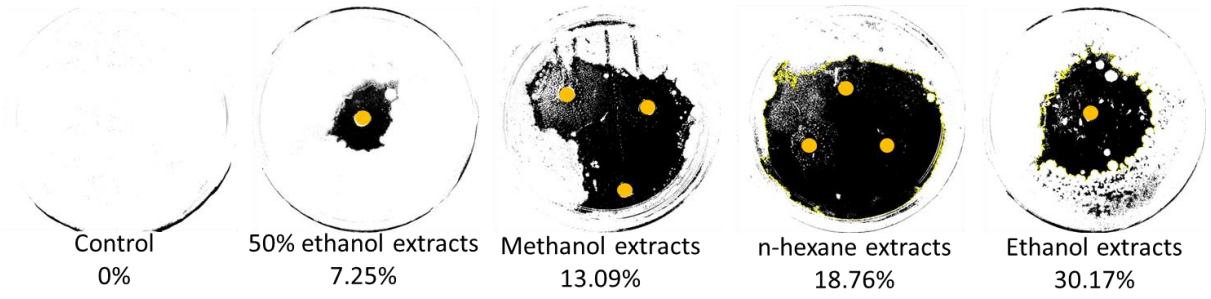

Fig. 3: Antimicrobial properties of essential oil from *N. sativa*

References

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